

CLAIMS:

1. An isolated nucleic acid encoding a Yellow Fever virus comprising a nucleic acid sequence that requires alteration of at least two nucleotides of a codon encoding amino acid 28 of an envelope protein to encode a glycine at amino acid 28 of the envelope protein.
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2. The nucleic acid of claim 1, further comprising a nucleic acid sequence that requires alteration of at least two nucleotides of a codon encoding amino acid 27 of an envelope protein to encode a histidine at amino acid 27 of the envelope protein.
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3. The nucleic acid of claim 1, further comprising a nucleic acid sequence that requires alteration of at least two nucleotides of a codon encoding amino acid 323 of an envelope protein to encode an arginine at amino acid 323 of the envelope protein.
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4. The nucleic acid of claim 1, further comprising a nucleic acid sequence that requires alteration of at least two nucleotides of a codon encoding amino acid 331 of an envelope protein to encode an arginine at amino acid 331 of the envelope protein.
- 20 5. The nucleic acid of claim 1, further comprising a nucleic acid sequence that requires alteration of at least two nucleotides of a codon encoding amino acid 48 of a NS2A protein to encode an alanine at amino acid 48 of the NS2A protein.
6. The nucleic acid of claim 1, further comprising a nucleic acid sequence that requires alteration of at least two nucleotides of a codon encoding amino acid 98 of a NS4B protein to encode an isoleucine at amino acid 98 of the NS4B protein.
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7. The nucleic acid of claim 2, further comprising a nucleic acid sequence that requires alteration of at least two nucleotides of a codon encoding amino acid 323 of an envelope protein to encode an arginine at amino acid 323 of the envelope protein.
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8. The nucleic acid of claim 2, further comprising a nucleic acid sequence that requires alteration of at least two nucleotides of a codon encoding amino acid 331 of an envelope protein to encode an arginine at amino acid 331 of the envelope protein.
- 5 9. The nucleic acid of claim 2, further comprising a nucleic acid sequence that requires alteration of at least two nucleotides of a codon encoding amino acid 48 of a NS2A protein to encode an alanine at amino acid 48 of the NS2A protein.
- 10 10. The nucleic acid of claim 2, further comprising a nucleic acid sequence that requires alteration of at least two nucleotides of a codon encoding amino acid 98 of a NS4B protein to encode an isoleucine at amino acid 98 of the NS4B protein.
- 15 11. The nucleic acid of claim 3, further comprising a nucleic acid sequence that requires alteration of at least two nucleotides of a codon encoding amino acid 331 of an envelope protein to encode an arginine at amino acid 331 of the envelope protein.
- 20 12. The nucleic acid of claim 3, further comprising a nucleic acid sequence that requires alteration of at least two nucleotides of a codon encoding amino acid 48 of a NS2A protein to encode an alanine at amino acid 48 of the NS2A protein.
- 25 13. The nucleic acid of claim 3, further comprising a nucleic acid sequence that requires alteration of at least two nucleotides of a codon encoding amino acid 98 of a NS4B protein to encode an isoleucine at amino acid 98 of the NS4B protein.
- 30 14. The nucleic acid of claim 4, further comprising a nucleic acid sequence that requires alteration of at least two nucleotides of a codon encoding amino acid 48 of a NS2A protein to encode an alanine at amino acid 48 of the NS2A protein.
15. The nucleic acid of claim 4, further comprising a nucleic acid sequence that requires alteration of at least two nucleotides of a codon encoding amino acid 98 of a NS4B protein to encode an isoleucine at amino acid 98 of the NS4B protein.

16. The nucleic acid of claim 5, further comprising a nucleic acid sequence that requires alteration of at least two nucleotides of a codon encoding amino acid 98 of a NS4B protein to encode an isoleucine at amino acid 98 of the NS4B protein.

5 17. An isolated nucleic acid encoding a Yellow Fever virus with a viral genome that comprises at least one of the following alterations:

- a) an alteration in the nucleic acid sequence resulting in an envelope protein with a histidine at amino acid 27;
- 10 b) an alteration in the nucleic acid sequence resulting in an envelope protein with a glycine at amino acid 28;
- c) an alteration in the nucleic acid sequence resulting in an envelope protein with a alanine at amino acid 155;
- d) an alteration in the nucleic acid sequence resulting in an envelope protein with an arginine at amino acid 323;
- 15 e) an alteration in the nucleic acid sequence resulting in an envelope protein with an arginine at amino acid 331;
- f) an alteration in the nucleic acid sequence resulting in a NS2A protein with an alanine at amino acid 48; or
- 20 g) an alteration in the nucleic acid sequence resulting in a NS4B protein with an isoleucine at amino acid 98.

18. The nucleic acid of claim 17, wherein the nucleic acid is RNA.

19. The nucleic acid of claim 17, wherein the nucleic acid is DNA.

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20. The nucleic acid of claim 17, wherein the viral genome comprises at least two of alterations a-g.

21. The nucleic acid of claim 17, wherein the viral genome comprises at least
30 three of alterations a-g.

22. The nucleic acid of claim 17, wherein the viral genome comprises at least four of alterations a-g.

23. The nucleic acid of claim 17, wherein the viral genome comprises at least five of alterations a-g.
- 5 24. The nucleic acid of claim 17, wherein the viral genome comprises at least six of alterations a-g.
25. The nucleic acid of claim 17, wherein the viral genome comprises seven of alterations a-g.
- 10 26. The nucleic acid of claim 17, wherein the nucleic acid has a nucleic acid sequence as set forth in SEQ ID NO:1.
- 15 27. A isolated nucleic acid comprising 10 to 200 contiguous nucleotides of SEQ ID NO:1.
28. The isolated nucleic acid of claim 27, wherein said nucleic acid comprises 15 to 150 contiguous nucleotides.
- 20 29. The isolated nucleic acid of claim 27, wherein said nucleic acid comprises 20 to 100 contiguous nucleotides.
30. The isolated nucleic acid of claim 27, wherein said nucleic acid comprises 25 to 50 contiguous nucleotides.
- 25 31. A vaccine composition comprising a Yellow Fever virus with a viral genome that comprises at least one of the following alterations:
- 30 a) an alteration in a nucleic acid sequence encoding amino acid 323 of an/the envelope protein, wherein the first alteration requires more than one nucleotide change to encode an arginine;
- b) an alteration in a nucleic acid sequence encoding amino acid 27 of an/the envelope protein, wherein the second alteration requires more than one nucleotide change to encode a histidine;

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- c) an alteration in a nucleic acid sequence encoding amino acid 28 of the envelope protein, wherein the second alteration requires more than one nucleotide change to encode a glycine;
- d) an alteration in a nucleic acid sequence encoding amino acid 155 of the envelope protein, wherein the second alteration requires more than one nucleotide change to encode an alanine;
- e) an alteration in a nucleic acid sequence encoding amino acid 331 of the envelope protein, wherein the second alteration requires more than one nucleotide change to encode an arginine;
- 10 f) an alteration in a nucleic acid sequence encoding amino acid 48 of the NS2A protein, wherein the second alteration requires more than one nucleotide change to encode an alanine; or
- g) an alteration in a nucleic acid sequence encoding amino acid 98 of the NS4B protein, wherein the second alteration requires more than one nucleotide change to encode an isoleucine.
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32. The vaccine composition of claim 31, wherein the viral genome comprises at least two of alterations a-g.

20 33. The vaccine composition of claim 31, wherein the viral genome comprises at least three of alterations a-g.

34. The vaccine composition of claim 31, wherein the viral genome comprises at least four of alterations a-g.

25 35. The vaccine composition of claim 31, wherein the viral genome comprises at least five of alterations a-g.

30 36. The vaccine composition of claim 31, wherein the viral genome comprises at least six of alterations a-g.

37. The vaccine composition of claim 31, wherein the viral genome comprises seven of alterations a-g.

38. The vaccine composition of claim 31, wherein the composition is a pharmaceutically acceptable formulation.

5 39. The vaccine composition of claim 31, wherein the Yellow Fever virus is a 17D virus.

40. The vaccine composition of claim 31, wherein the Yellow Fever virus is a 17D-204 virus.

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41. The vaccine composition of claim 31, wherein the Yellow Fever virus is a 17DD virus.

15 42. The vaccine composition of claim 31, wherein the viral genome comprises an alteration in a nucleic acid sequence encoding amino acid 28 of the envelope protein, wherein alteration of the sequence encoding amino acid 28 requires more than one nucleotide change to encode a glycine;

20 43. A method for producing an attenuated Yellow Fever virus comprising introducing into a Yellow Fever virus genome a missense mutation that would require two nucleotide changes to encode a supervirulence amino acid.

44. A method for producing a Yellow Fever virus vaccine comprising:
25 a) identifying a mutation that results in a missense mutation in a first Yellow Fever viral genome that is associated with an increased virulence of the virus;
b) modifying an attenuated Yellow Fever viral genome by mutation of a codon associated with the missense mutation resulting in a reduced probability of reversion to a virulent phenotype.

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45. The method of claim 44, wherein the missense mutation results in an envelope protein having an arginine at amino acid position 323.

46. The method of claim 44, wherein modifying the attenuated Yellow Fever virus is by substituting a second codon that encodes for a conservative amino acid change.

47. A method for identifying a compound active against a viral infection comprising:

- a) providing a virus expressed from a viral construct comprising a nucleic acid encoding a Yellow Fever virus comprising an envelope protein comprising an arginine at amino acid 323 or a glycine at amino acid 28;
- b) contacting the virus with a candidate substance; and
- c) comparing the infectious ability of the virus in the presence of said candidate substance with the infectious ability of the virus in a similar system in the absence of the candidate substance.

48. The method of claim 47, wherein the nucleic acid encodes a virus with an envelope protein further comprising an arginine at amino acid 323, a histidine at amino acid 27, a glycine at amino acid 28, an alanine at amino acid 155, and an arginine at amino acid 331.

49. The method of claim 47, wherein the nucleic acid sequence is that set forth in SEQ ID NO:1.

50. A method of vaccination against a virus comprising administering to a subject a Yellow Fever virus with a viral genome that comprises at least one of the following alterations:

- a) an alteration in the nucleic acid sequence encoding amino acid 323 of an envelope protein, wherein it requires more than one nucleotide change to encode an arginine;
- b) an alteration in the nucleic acid sequence encoding amino acid 27 of the envelope protein, wherein it requires more than one nucleotide change to encode a histidine;

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- c) an alteration in the nucleic acid sequence encoding amino acid 28 of the envelope protein, wherein it requires more than one nucleotide change to encode a glycine;
- d) an alteration in the nucleic acid sequence encoding amino acid 155 of the envelope protein, wherein it requires more than one nucleotide change to encode an alanine;
- e) an alteration in the nucleic acid sequence encoding amino acid 331 of the envelope protein, wherein it requires more than one nucleotide change to encode an arginine;
- 10 f) an alteration in the nucleic acid sequence encoding amino acid 48 of the NS2A protein, wherein it requires more than one nucleotide change to encode an alanine; or
- g) an alteration in the nucleic acid sequence encoding amino acid 98 of the NS4B protein, wherein it requires more than one nucleotide change to encode an isoleucine.
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51. The method of vaccination of claim 50, wherein the viral genome comprises at least two alterations.

20 52. The method of vaccination of claim 50, wherein the viral genome comprises at least three alterations.

53. The method of vaccination of claim 50, wherein the viral genome comprises at least four alterations.

25 54. The method of vaccination of claim 50, wherein the viral genome comprises at least five alterations.

55. The method of vaccination of claim 50, wherein the viral genome comprises at least six alterations.

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56. The method of vaccination of claim 50, wherein the viral genome comprises seven alterations.

57. The method of vaccination of claim 50, wherein the composition is a pharmaceutically acceptable formulation.

5 58. The method of vaccination of claim 50, wherein the Yellow Fever virus is a 17D virus.

59. The method of vaccination of claim 50, wherein the Yellow Fever virus is a 17D-204 virus.

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60. The method of vaccination of claim 50, wherein the Yellow Fever virus is a 17DD virus.